claims and the amendments introduce no new matter. Support is replete throughout the specification (*e.g.*, page 13, lines 29-30, page 26, lines 11-13, page 26, lines 25-28, *etc.*),

Restriction requirement.

Pursuant to a restriction requirement made final, Applicants cancel claims 18-50 with entry of this amendment. Please note, however, that Applicants reserve the right to file subsequent applications claiming the canceled subject matter and the claim cancellations should not be construed as abandonment or agreement with the Examiner's position in the Office Action.

Drawings.

Applicants note that the application was filed with informal drawings and that formal drawings will be required when the application is allowed.

Objections to the specification.

The Examiner objected to the trademarks Tween 20 at page 13, line 2, "Bio-Rad' at page 48, line 27, and "Lipofectamine" at page 61, line 6. Per the Examiner's request, the specification is amended to capitalize these terms

35 U.S.C. §112, Second Paragraph.

Claims 1-17 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite for the following reasons:

1) The recitation in claim 1 of the phrase "antibody binding moieties";
2) The recitation in claim 1 "contacting cells with members of phage display library"; and
3) The recitation in claim 1 of "can be" and "if bound";

Claim 1 is amended herein to eliminate the phrase "polypeptide or antibody binding moieties", the phrase "can be", and the phrase "if bound" thereby obviating these rejections.

35 U.S.C. §103(a).

Claims 1-17 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of WO 92/20791 ((Winter *et al.*) in view of either Ewijk *et al.* (1997) *Proc. Natl. Acad. Sci., USA,* 94: 3903-

3908 or Stausbol-Gron *et al.* (1996) *FEBS Letts.*, 39: 71-75). In addition, claims 1-17 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Barry *et al.* (1996) *Nature Medicine* 2(3): 299-305, in view of either Ewijk *et al.* or Stausbol-Gron *et al.* Claims 1-17 were also rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Larocca *et al.* (U.S. Patent 6,054,312) in view of either Ewijk *et al.* or Stausbol-Gron *et al.*

In particular, the Examiner alleges that Winter *et al.*, Barry *et al.*, and Larocca *et al.* teach the use of phage display libraries to select phage that bind particular cells. The Examiner however, admits that neither Winter *et al.*, nor Barry *et al.*, *nor Larocca et al.* teach the use of a subtractive cell line to remove the non-specifically bound phage particles. The Examiner then alleges that either Ewijk *et al.* or Stausbol-Gron *et al.* teach a phage display subtraction method. Applicants respectfully traverse.

The Examiner is reminded that prima facie case of obviousness requires that the combination of the cited art, taken with general knowledge in the field, must provide all of the elements of the claimed invention. When a rejection depends on a combination of prior art references, there must be some teaching, suggestion, or motivation to combine the references. *In re Geiger,* 815 2 USPQ2d 1276, 1278 (Fed. Cir. 1987). Moreover, to support an obviousness rejection, the cited references must additionally provide a reasonable expectation of success. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991), *citing In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988).

In the instant case, the combination of references cited by the Examine fail to teach or suggest all of the elements of the presently claimed invention. Moreover, combination of Stausbol-Gron *et al.* or Ewijk *et al.* with any of the primary references would result in a screening system unsuited to the detection of internalizing polypeptides. Accordingly, the cited art offers no motivation to combine the references are proposed by the Examiner.

A) The cited references fail to teach or suggest the presently claimed methods.

The cited art fails to teach or suggest the presently claimed invention. The present invention provides a simple and efficient method of identifying of selecting a polypeptide that is internalized into a target cell. The method involves contacting the target cell(s) with a phage display library, using a **strong wash** to remove external phage that are tightly or weakly bound and identifying

internalized members of the library. In certain embodiments, the target cells are contacted with **live cells** of a subtractive cell line.

The cited art fails to teach or suggest the use of a strong wash to remove the undesired phage and identification/selection of the remaining internalized phage. The cited art also fails to teach or suggest the use of a subtractive cell line comprising cells, particularly living cells in combination with a strong wash.

i) Stausbol-Gron fails to teach a subtractive cell line or a strong wash.

Contrary to the Examiner's assertion, Stausbol-Gron fails to teach a subtractive cell line or a strong wash. Stausbol-Gron teaches the use of a competitive two solid phage system for selecting phage. The system uses competitive proteins in solution or on the walls of an immunotube. There is no teaching or suggestion of a subtractive cell line comprising living cells.

Moreover, Stausbol-Gron teaches wash conditions that utilize a weak wash (phosphate buffered saline). Thus Stausbol-Gron expressly states:

> In the competitive two solid phase system, the target proteins, MIX + LDH proteins or FM55p **proteins were coated on immunobead(s)**. The second solid phase support was an **immunotube coated with competitive MIX proteins or competitive FM55p proteins, respectively**. Moreover, the **competitive proteins were added in solution**. [emphasis added] (Figure 1 legend).
>
> The immunobead was washed 20 times in **PBS with 0.2% Tween-20, and 20 times in PBS**. [emphasis added] page 72, column 1)
>
> * * *
>
> The immmunobeads were then washed by soaking 5 times for 2 min in PBS with 0.1% Tween-20, once for 10 min in PBS with 0.1% Tween-20, and 3 times for 2 min in PBS, and the bound phage were eluted and propagated as described above. [emphasis added] page 72, column 1).

In addition, Stausbol-Gron teaches the selection/use of the phage that are eluted off of the target (immunobeads), while the presently claimed methods contemplate selection/use of the phage that are retained (*i.e.,* internalized) by the target cells. Thus, in contrast to the present invention Stausbol-Gron expressly teaches:

> The bound phage were eluted with 1 ml 100 M triethylamine for 10 min at room temperature and neutralized with 0.5 ml 1 M Tris, **pH.7.4**. Exponentially growing TG-1 bacteria **were infected with 1 ml of the eluate for 30 min at 37°C, and phage were produced by super-infection** with the helper phage VCS-M13 (Stragagene) and growing the bacteria with shaking overnight at 30°C. [emphasis added] (page 72, column 1)

Stausbol-Gron thus teaches the use of a "weak wash" (PBS), subtraction using isolated proteins in solution or attached to an immunotube, and selection of the phage washed off of the target. Stausbol-Gron fails to teach the use of a strong wash, of a subtractive cell line comprising living cells, or the selection of phage retained by the target immunobeads. The combination of Stausbol-Gron with Winter *et al.,* Barry *et al.,* or Larocca *et al.* simply fails to render the presently claimed invention obvious and the rejection under 35 U.S.C. §103(a) on these grounds should be withdrawn.

ii) Stausbol-Gron teaches away from a strong wash.

Stausbol-Gron actually teaches away from the use of a strong wash. In particular, this reference expressly states:

> The dilemma remains, however, that **attempts to improve the efficiency of selection inhibition by using higher stringency during the panning rounds will tend to decrease the diversity in the subtracted phage pool, because low affinity binders are lost [33]. Consequently, a less stringent approach may be more suitable** in some cases. [emphasis added] (page 74, column 2)

Stausbol-Gron thus expressly states that a strong wash (*i.e.*, high stringency) can decrease the diversity of the phage pool by eliminating low affinity binders and suggests that lower stringency is desirable. In contrast, the presently claimed methods pertain to the use of a strong wash (*e.g.* high stringency). Contrary to Stausbol-Gron, in the presently claimed methods, it is desired to

eliminate weak binders. Indeed, it is desirable to eliminate strong binders that are not internalized into the target cells. Thus, the present specification expressly states:

> A second strong washing step is preferably used after internalization of members of the phage display library. **The "strong" washing step is intended to remove tightly- and weakly-bound surface phage.** [emphasis added] (specification, page 26, lines 25-28)

Stausbol-Gron thus expressly teaches away from the presently claimed methods. Again, the combination of Stausbol-Gron with Winter *et al.*, Barry *et al.*, or Larocca *et al.* simply fails to render the presently claimed invention obvious and the rejection under 35 U.S.C. §103(a) on these grounds should be withdrawn.

iii) The defects of Stausbol-Gron are not remedied by Ewijk *et al.*

Ewijk *et al.* also fails to teach the use of a strong wash to eliminate the undesired/unselected phage. Rather, Ewijk *et al.* teaches the use of a "weak wash" for phage selection:

> The following day thymic fragments were allowed to sediment, the supernatant was decanted, and the thymic fragments were vigorously rinsed, using a total volume of 2 linters of M-PBS containing 0,05% Tween 20 (M-PB-Tw), **to remove nonspecifically adhered phages**. [emphasis added] (page 3904, column 1).

Ewijk *et al.* then utilizes use a strong wash (pH 2.5) to elute and re-screen the desired phage rather than to remove and eliminate undesired phage:

> To elute specifically bound phages, thymic fragments were transferred in a volume of 300 μl of M-PBS-Tw to a 15 –ml tube containing 450 μl of sodium citrate (**pH 2.5**). After 5 min, the pH was neutralized by adding 375 μl of 1 M Tris-HCl buffer (pH 7.4). Finally, 3 ml of 2TY medium (GIBCO/BRL) and 3 ml of Escherichia coli XL-1 blue (Stratagene) was added. Infection was allowed to proceed for 30 minutes. Bacteria were centrifuged at 2,2000 Xg for 30 min, suspended in 0.5 ml of 2TY, and plated on agar plates containing 25 μg/ml tetracycline, 100 μg/ml ampicillin, and 55 glucose (TAG) After overnight culture at 37°C, plates were scraped and bacteria were frozen in stock vials **or used to prepare the next library as describe in detail elsewhere(13)**. [emphasis added] (page 3904, column 1).

Ewijk *et al.* thus discloses a method that is essentially the opposite of the presently claimed method. Ewijk *et al.* teaches the use of a weak wash to screen for binding phage and the use of a strong wash to elute and recover the desired binders. In contrast, the presently claimed method contemplates the use of a strong wash to eliminate all externally bound phage including those that specifically bind to the target cells. The remaining **internalized** phage are then recovered. Ewijk *et al.* thus leads one of skill away from the presently claimed methods.

Ewijk *et al.* also fails to teach or suggest a subtraction with a subtractive cell line comprising **living cells**. To the contrary, Ewijk *et al.* expressly teaches the use of fixed (dead cells:

> To prepare stromal cells for phage selection, **we mildly fixed thymic tissue** with a solution of **0.05% gluteraldehyde** (Polysciences) in PBS using the total body perfusion fixation (16). [emphasis added] (page 3904, column 1)
>
> * * *
>
> To this solution, 1 ml of M-PBS, containing 2.5 x 10^8 005% **gluteraldehyde-fixed** adsorber cells (thymocytes + spleen cells), was added and allowed to incubate for 1 hr at room temperature. [emphasis added] (page 3904, column 1)

Ewijk *et al.* thus fails to teach the use of a strong wash to eliminate the undesired phage or to tech the use of a live cell line. Thus, Ewijk *et al.* taken with any or all of the primary references fails to teach or suggest the presently claimed invention. Accordingly, the rejection under 35 U.S.C. §103(a) on these grounds should be withdrawn.

iv) Ewijk *et al.* teaches away from the presently claimed invention.

Moreover, Ewijk *et al.* teaches away from the presently claimed invention. Ewijk *et al.* teaches a method for detecting surface antigen, not internalizing polypeptides. Thus Ewijk *et al.* expressly states:

> **Although our selection method was aimed to isolate MoPhabs directed to cell surface determinants expressed on intact thymic stromal cells,** several of the isolated MoPHabs recognized molecules expressed in the cytoplasm of stromal cells. This result may be inherent n the present procedure: after fixation, the thymus was cut into fragments thus exposing cytoplasmic determinants.

In addition, because Ewijk *et al.* teaches the use of fixed (dead) cells as target cells, the reference leads one of skill away from methods that can be used to detect internalizing polypeptides. Ewijk *et al.* thus leads one of skill away from the presently claimed invention consequently **fails** to support a *prima facie* case of obviousness.

v. Barry *et al.* teaches away from the presently claimed invention.

Barry *et al.* also teaches away from the present invention. Claim 1, as amended herein clarifies that the strong wash is used to remove and eliminate phage attached to the surface of the target cells. The removed phage are thus eliminated from further screening steps facilitating selection of internalizing phage.

This is in contrast to the methods disclosed by Barry *et al.* Barry *et al* expressly teaches recovering the phage removed from the target cell surface and using those phage in subsequent panning rounds:

> Binding was conducted at 4° C to avoid endocytosis of the phage. Ten library equivalents of the 12-amino acid polymer (12-mer) library (3×10^9 phage from ON159.3) were incubated on the cells, the cells were washed, and **the acid-labile phage were eluted from the cell surface and recovered as the acid-eluted fraction**. In the first round of selection, approximately 10^{-6} of the input phage were eluted by acid (data not shown). Although peptide-presenting phage are usually recovered from their target molecule by using alow pH wash[4,5], slightly more phage remained associated with the cells following multiple acid washes than were eluted by the acid. **This cell associated fraction was also recovered and amplified because** these phage might have higher affinities for the cells or involve hydrophobic interactions. [emphasis added] (page 299, column 2)

This is further illustrated in Figure 1*b* which illustrates the recycling (rescreening) of the acid-eluted fraction of phage.

Barry *et al.* thus expressly leads one of skill away from a method in which the phage that bind the target cell surface are removed and eliminated and therefore leads one of skill away from the presently claimed invention. Accordingly, Barry *et al.* fails to support a *prima facie* case of obviousness and the rejection in light of Barry *et al* t in view of either Ewijk *et al.* or Stausbol-Gron *et al.* should be withdrawn.

B) The cited references offer no motivation to combine.

The Examiner is also reminded that the MPEP expressly states that "if the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification." MPEP §2143.01, *citing In re Gordon*, 221 USPQ 1125 (Fed. Cir. 1984).

In the instant case, the combination of the methods of Stausbol-Gron or Ewijk *et al.* with the primary references (Winter *et al.*, Barry *et al.*, and/or Larocca *et al.*) would produce systems unsatisfactory for the detection/selection of internalizing polypeptides and thus offer no motivation to make the modification(s) proposed by the Examiner.

i) Stausbol-Gron teaches the use of cell-free extracts.

Stausbol-Gron, *et al.* teaches the use of a two solid phase system comprising target protein(s) (antigen) attached to immunobeads and competitive proteins attached to an immunotube and in solution (*see* Figure 1, page 72). The reference offers no teaching or suggestion of the use of an intact cell. Use of the subtractive procedure taught by Stausbol-Gron *et al.* with the methods of Winter *et al.*, Barry *et al.*, and/or Larocca *et al.*, would result in a cell-free screening system. **Lacking target cells, such a cell-free system cannot be used to identify internalizing antibodies.**

Modification of Winter *et al.*, Barry *et al.*, and/or Larocca *et al.* with the subtractive methods disclosed by Stausbol-Gron *et al.* would render the art unsatisfactory for its intended purpose. Thus, as recognized in *In re Gordon,* and stated in MPEP §2143.01, there is no suggestion or motivation to make the proposed modification. Accordingly, the combination of Stausbol-Gron *et al.* with Winter *et al.*, Barry *et al.*, and/or Larocca *et al.* does not support a *prima facie* case of obviousness and the rejection on these grounds should be withdrawn.

ii) Ewijk *et al.* teaches the use of fixed (dead) cells.

Ewijk *et al.* teaches the use of fixed (dead cells) as both "target cells" and to readsorb a library. The fixed target cells are dead and hence, incapable of internalizing a binding moiety. Modification of Winter *et al.*, Barry *et al.*, and/or Larocca *et al.* with the methods disclosed by Ewijk *et al.* would produce a screening system using fixed cells that could not be used to screen for

internalizing polypeptides. Accordingly, the combination of Ewijk *et al.* with Winter *et al.*, Barry *et al.*, and/or Larocca *et al.* does not support a *prima facie* case of obviousness and the rejection on these grounds should be withdrawn.

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-7871.

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Respectfully submitted,

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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE IN 09/249,529 WITH ENTRY OF THIS AMENDMENT

In the specification:

Page 12, line 26 through page 13:

The following abbreviations are used herein: AMP, ampicillin; c-erbB-2 ECD, extracellular domain of c-erbB-2; CDR, complementarity determining region; ELISA, enzyme linked immunosorbent assay; FACS, fluorescence activated cell sorter; FR, framework region; Glu, glucose; HBS, hepes buffered saline, 10 mM hepes, 150 mM NaCl, pH 7.4; IMAC, immobilized metal affinity chromatography; k_{on}, association rate constant; k_{off}, dissociation rate constant; MPBS, skimmed milk powder in PBS; MTPBS, skimmed milk powder in TPBS; PBS, phosphate buffered saline, 25 mM NaH_2PO_4, 125 mM NaCl, pH 7.0; PCR, polymerase chain reaction; RU, resonance units; scFv or scFv, single-chain Fv fragment; TPBS, 0.05% v/v **[Tween]** **TWEEN®** 20 in PBS; SPR, surface plasmon resonance; V_k, immunoglobulin kappa light chain variable region; V_λ, immunoglobulin lambda light chain variable region; V_L, immunoglobulin light chain variable region; V_H, immunoglobulin heavy chain variable region; wt, wild type.

Page 48, lines 12-29:

SKBR3 cells were grown on coverslips in 6-well culture plates (Falcon) to 50% of confluency. Culture medium was renewed 2 hours prior to the addition of 5.10^{11} cfu/ml of phage preparation (the phage preparation representing a maximum of 1/10 of the culture medium volume) or 20 µg/ml of purified scFv or diabody in phosphate buffered saline, pH 7.4 (PBS). After 2 hours of incubation at 37°C, the wells were quickly washed 6 times with ice cold PBS and 3 times for 10 minutes each with 4 mL of stripping buffer (50 mM glycine pH 2.8, 0.5 M NaCl, 2M urea, 2% polyvinylpyrrolidone) at RT. After 2 additional PBS washes, the cells were fixed in 4% paraformaldehyde (10 minutes at RT), washed with PBS, permeabilized with acetone at -20°C (30 seconds) and washed again with PBS. The coverslips were saturated with PBS-1% BSA (20 min. at RT). Phage particles were detected with biotinylated anti-M13 immunoglobulins (5 Prime-3 Prime, Inc, diluted 300 times) (45 min. at RT) and Texas red-conjugated streptavidin (Amersham, diluted 300

times) (20 min. at RT). Soluble scFv and diabodies containing a C-terminal myc peptide tag were detected with the mouse mAb 9E10 (Santa Cruz Biotech, diluted 100 times) (45 min. at RT), anti-mouse biotinylated immunoglobulins (Amersham, diluted 100 times) and Texas red-conjugated streptavidin. Optical confocal sections were taken using a **[Bio-Rad]** **BIO-RAD®** MRC 1024 scanning laser confocal microscope. Alternatively, slides were analyzed with a Zeiss Axioskop UV fluorescent microscope.

Page 61, lines 4-12:

SKBR3 and MCF7 were grown in RPMI complemented with 10% fetal bovine serum (FBS) (Hyclone). 50 % confluent SKBR3 cells grown in 6-well plates were transfected with 1 μg of DNA per well using **[Lipofectamine]** **LIPOFECTAMINE®** (GIBCO BRL) as recommended by the manufacturer. pN2EGFP dsDNA was prepared by alkaline lysis using the Maxiprep Qiagen Kit (Qiagen Inc.). ssDNA was extracted from 500 μl of phagemid preparation (see below) by 2 phenol extractions followed by ethanol precipitation. DNA was quantified by spectophotometry with 1.0 A_{260} nm equal to 40 μg/ml for ssDNA or 50 μg/ml for dsDNA. For GFP detection, cells were detached using a trypsin-EDTA mix (GIBCO BRL) and analyzed on a FACScan (Becton Dickinson).

In the claims:

1. A method of selecting a polypeptide **that is internalized into a target cell [or antibody binding moieties that are internalized into target cells]**, said method comprising:

i) contacting one or more [of said] target cells with one or more members of a phage display library **displaying one or more polypeptides;**

[ii) contacting members of said phage display library with a cells of a subtractive cell line;

iii) washing said target cells to remove said cells of a subtractive cell line and to remove members of said phage display library that are non-specifically bound or weakly bound to said target cells;]

ii) removing and eliminating members of said library that are bound to the exterior surface of said cells with a strong wash; and

[iv) culturing said target cells under conditions where members of said phage display library can be internalized if bound to an internalizing marker; and]

[v)] iii) identifying internalized members of said phage display library **that [if members of said phage display library]** are internalized into one or more of said target cells.

4. The method of claim 1, wherein said identifying comprises recovering internalized phage and repeating steps (i) through **[(v)] (iii)** to further select for internalizing binding moieties.

11. The method of claim 1, wherein step (ii) is performed at about 4°C**[and step (iv) is performed at about 37°C].**

51. The method of claim 1, wherein said method further comprises contacting the target cells with cells of a subtractive cell line.

52. The method of claim 51, wherein said method further comprises contacting the target cells with live cells of a subtractive cell line.

53. The method of claim 1, wherein said removing comprises contacting the target cells with a low pH wash.

54. The method of claim 51, wherein said removing comprises contacting the target cells with a low pH wash.

55. The method of claim 1, wherein said removing comprises contacting the target cells with a trypsin.

56. The method of claim 51, wherein said removing comprises contacting the target cells with a trypsin.

57. The method of claim 51, wherein the target cells are cells that are transformed a nucleic acid that encodes and expresses a target receptor and the subtractive cell line is the non-transformed cell line.